Compound Heterozygosity for Silent and Dominant Glycine Substitution Mutations in COL7A1 Leads to a Marked Transient Intracytoplasmic Retention of Procollagen VII and a Moderately Severe Dystrophic Epidermolysis Bullosa Phenotype

To the Editor:

Dystrophic epidermolysis bullosa (DEB) is caused by mutations in the COL7A1 gene encoding collagen VII (Uitto and Christiano, 1994; Hovnanian *et al*, 1997; Hammami-Hauasli *et al*, 1998b), the major component of anchoring fibrils. In autosomal dominant and recessive DEB, different phenotypes are caused by heterozygous, compound heterozygous, or homozygous mutations in COL7A1. Premature termination codon mutations in both alleles lead to the most severe phenotype, the Hallopeau-Siemens subtype, with lack of expression of collagen VII (Uitto and Christiano, 1994; Hovnanian *et al*, 1997).

Among the different mutations, substitution of a glycine residue in the triple helical collagenous domain by other, bulkier amino acids present a biologically intriguing situation. Glycine substitutions associated with dominant DEB interfere with folding and secretion of collagen VII in a dominant negative manner (Christiano and Uitto, 1996; Hammami-Hauasli *et al*, 1998b); however, when combined with a normal allele, certain heterozygous glycine substitutions do not interfere with the biosynthesis of collagen VII in this way and thus do not lead to a clinical phenotype (Christiano *et al*, 1996; Shimizu *et al*, 1996; Hovnanian *et al*, 1997). These mutations were termed silent glycine substitutions. Combination of the silent glycine substitutions and different COL7A1 mutations in the other allele have been

Figure 1. Moderately severe DEB phenotype in the proband, and big toe nail dystrophy in individuals with maternal dominant glycine substitution mutation in COL7A1. The proband, a now 10-y-old girl (no. 2 in **Fig. 3**) who is compound heterozygous for the COL7A1 mutations G2316R and G2287R, demonstrated large scarring and ulcerating plaques, loss of all the toe and finger nails, and mild fusion of left toes (*A–E*). Her mother (*G*: no. 4 in **Fig. 3**), her maternal uncle (*H*: no. 5 in **Fig. 3**), and her maternal grandmother (*I*: no. 6 in **Fig. 3**), who are heterozygous for G2287R, showed nail deformity restricted to big toes without any sings of skin fragility. All other family members without this G2287R mutation have no signs of nail dystrophy, including the father (*F*: no. 1 in **Fig. 3**) and the sister (no. 3 in **Fig. 3**) of the patient who are heterozygous for G2316R silent glycine substitution.

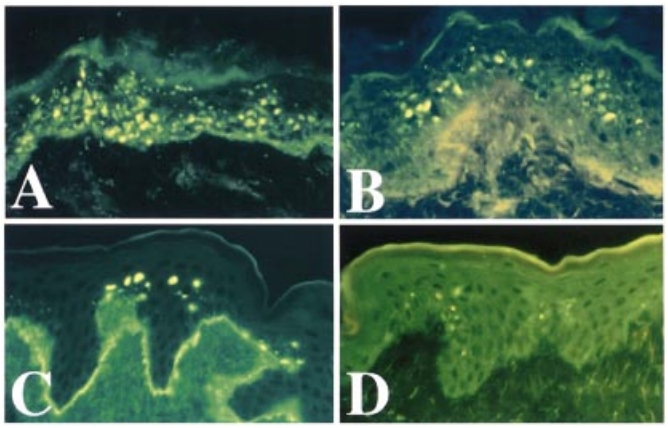

Figure 2. Procollagen VII was abundantly accumulated in the intracytoplasm of epidermis at the age of 3 d. In the skin at 3 d of age, abundant intracytoplasmic accumulation and weak linear BMZ distribution of collagen VII was observed with antibodies to the NC-1 domain and to the triple helical domain (*A*). While BMZ was negative with antibodies to the NC-2 domain, the abundant intracytoplasmic retention was clearly positive (*B*), indicating that most of the retained collagen was procollagen VII in which cleavage of NC-2 domain had not occurred. In the skin at the age of 10 y, bright linear labeling of NC-1 and triple helical domain was found along BMZ, with an intensity comparable with the normal control (*C*). In addition, careful observation disclosed infrequent punctuate labeling within the epidermis, though far less quantity compared with that of 3 d of age (*C*). The antibodies to the NC-2 domain showed completely negative labeling at BMZ, whereas the small infrequent punctuate material within the epidermis was positive (*D*), indicating that the labeling was not background, but indeed represented accumulation of procollagen VII.

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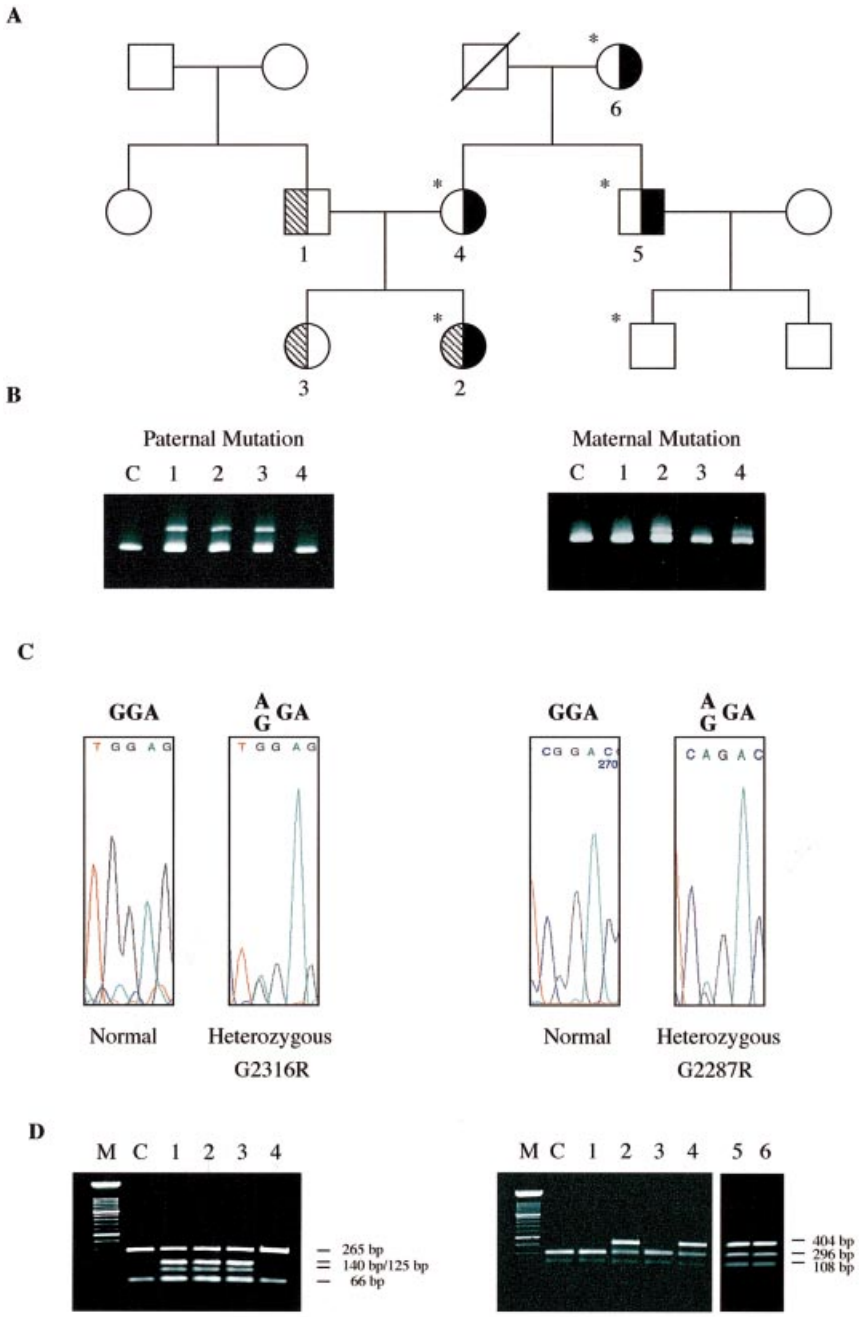

Figure 3. Identification of mutations G2316R and G2287R in COL7A1. (*A*) Pedigree. The black areas indicate the presence of the maternal mutation and the shaded areas the presence of the paternal mutation. The asterisk indicates family members with dystrophic toe nails. (*B*) Conformation-sensitive gel electrophoresis revealed heteroduplex bands in the polymerase chain reaction products spanning exon 89 of individuals 1, 2, and 3, and in the polymerase chain reaction products including exon 87 of probands 2 and 4. (*C*) Direct sequencing of the polymerase chain reaction products disclosed a heterozygous G→A transition in exon 89 in patient 2, her unaffected father 1, and her unaffected sister 3. This sequence alteration led to the glycine substitution G2316R (*left panels*). The dominant maternal mutation was a heterozygous G→A transition in exon 87, which led to the glycine substitution G2287R (*right panels*). (*D*) The paternal mutation created a *Mae* I site, which cleaved the 331 bp polymerase chain reaction product into two additional fragments of 140 bp and 125 bp (*left panel*). The maternal mutation G2287R was verified by the loss of an *Ava* II site, which led to the appearance of an uncleaved 404 bp fragment. This mutation could also be found in the family members 5 and 6 (*right panels*). Genomic DNA of the elder son of family member 5 was not available for testing.

shown to lead to various DEB phenotypes (Shimizu *et al*, 1996; Hammami-Hauasli *et al*, 1998a; Terracina *et al*, 1998). In this study, we explored the molecular basis of unusual DEB phenotype similar to transient bullous dermolysis of the newborn (TBDN) (Hashimoto *et al*, 1985).

The proband, a 10-year-old girl was born in September 1988 as the second child of nonconsanguineous healthy parents of Japanese ethnic origin as described previously (Hatta *et al*, 1995). At birth she presented with blisters on the entire integument and on mucous membranes. Although the severity of the skin condition has slightly decreased, the blister formation and deformities of the nails have continued since then. At the age of 10, she had large scarring and ulcerated plaques and lost all her nails, and deformity of the left toes had developed (**Fig 1*A–E***).

In order to study the expression and processing of procollagen VII in the patient's skin at the age of 3 d, 4 y, and 10 y, we used three domain specific antibodies for indirect immunofluorescence staining. Antibodies against the NC-1 (Leigh *et al*, 1987) and the triple-helical domain (Bruckner-Tuderman *et al*, 1987) recognize both procollagen VII and mature collagen VII. In contrast, antibodies against the carboxyterminal NC-2 domain (Bruckner-Tuderman *et al*, 1995) react only with procollagen VII, which has not yet been processed to mature collagen. At 3 d of age, abundant intracytoplasmic accumulation and weak linear BMZ distribution of collagen VII was observed in the proband's skin with antibodies to the NC-1 domain and to the triple helical domain. While BMZ was negative with antibodies to the NC-2 domain, the abundant intracytoplasmically retained material was clearly positive, indicating that most of the accumulating molecules were immature procollagen VII still possessing the NC-2 domain (**Fig 2**). In the skin at the age 4 y and 10 y, bright linear labeling with antibodies to the NC-1 and the triple helical domains was seen along BMZ. In addition, infrequent punctuate labeling within the epidermis was

positive with antibody to NC-2 domain, indicating that this corresponded to residual accumulation of procollagen VII (**Fig 2**).

For mutation detection, genomic DNA from the patient and her family members (**Fig 3**) were subjected to polymerase chain reaction amplification and heteroduplex analysis and dideoxynucleotide sequencing. The mutation screening identified a heterozygous 6946G→A transition in exon 89 and a heterozygous 6859G→A transition in exon 87 (**Fig 3**). Both transitions substitute Arg for Gly and were designated as G2316R and G2287R. The paternal mutation G2316R, also found in the sister's DNA, created a *Mae* I site (**Fig 3*D*,** *left*). The maternal mutation G2287R eliminated an *Ava* II site (**Fig 3*D*,** *right*). These sequence variations in exons 87 and 89 were not found in 150 normal chromosomes, indicating that they did not represent neutral polymorphisms. Screening of the entire coding sequence and of all intron-exon borders of the COL7A1 gene (Christiano *et al*, 1997) (Gene bank accession no. L23982) did not reveal other nucleotide variations predicting changes in collagen VII polypeptide sequence.

Although the mother was described as clinically unaffected according to the previous report (Hatta *et al*, 1995), careful clinical re-examination of all the family members disclosed the presence of mild nail dystrophy restricted to both big toes, without skin fragility, only in individuals heterozygous for G2287R. These included the mother (**Fig 1*G***: no. 4 in **Fig 3**), the maternal uncle (**Fig 1*H***: no. 5 in **Fig 3**), and the maternal grandmother (**Fig 1*I***: no. 6 in **Fig 3**) of the index patient. Other family members without the G2287R mutation did not show any sings of nail deformities or skin fragility. Therefore, G2287R was interpreted as a dominantly inherited glycine substitution mutation leading to a very mild phenotype, which was overlooked at the time of the previous case report of this patient (Hatta *et al*, 1995). Individuals carrying the paternal mutation G2316R were clinically unaffected.

Recently we identified another dominant glycine substitution mutation in COL7A1, G2251E, which caused only toe nail dystrophy but not skin blistering when combined with a normal allele (Hammami-Hauasli *et al*, 1998a); however, when compound heterozygous with another silent glycine substitution in the daughter of the index patient, the mutation led to an extensive TBDN phenotype at birth. These results suggest the possibility that some individuals who have been diagnosed as suffering from idiopathic toe nail dystrophy of unknown origin without skin fragility, might have been carriers of certain glycine substitution mutations of COL7A1, such as G2251E or G2287R.

There are only two previously reported cases with TBDN in which molecular defects have been identified. One is a COL7A1 splice site mutation in a TBDN child in a family with a dominant pedigree (Christiano *et al*, 1997a). The second case is a combination of silent glycine substitution (G1519D) and dominant glycine substitution mutations (G2251E) (Hammami-Hauasli *et al*, 1998a), a similar constellation as seen in the present proband. The morphologic hallmark of TBDN, marked transient accumulation of procollagen VII might represent mutant, slowly secreted collagen VII molecules in postnatal skin where the rate of collagen synthesis is high. Accordingly, transitory blistering in TBDN has possibly more to do with the quantity rather than quality of the abnormal collagen VII molecules. Interestingly, the cessation of blister formation in TBDN roughly coincides with a reduction in the biosynthesis rate and the normalization of collagen VII deposition at the dermo–epidermal junction, which is likely to indicate deposition of sufficiently functional anchoring fibrils (Hammami-Hauasli *et al*, 1998a). Although a combination of silent and dominant glycine substitution mutations was also found in the above case with TBDN (Hammami-Hauasli *et al*, 1998a), this is the first case in which these combinations were shown to be associated with a moderately severe classical DEB phenotype with marked transient epidermal retention of procollagen VII at birth.

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